SEQUENCE LISTING

<110> Institut National de la Santé et de la Recherche Médicale -INSERM ICHAS Francois DE GIORGI ICHAS Francesca PIAZZA Pier-Vincenzo DESSOLIN Jean SCHEMBRI Laura TOMASELLO Flora LARTIGUE Lydia <120> Method for demonstration of a molecular event in a cell by means of fluorescent marker proteins <130> D20600 <140> PCT/FR2004/001678 <141> 2004 - 06 - 30 <150> FR 03/08 186 <151> 2003-07-04 <160> 14 <170> PatentIn version 3.2 <210> 1 <211> 195 <212> DNA <213> Artificial sequence <220> <223> Probe <220> <221> CDS <222> (1)..(174) <400> 1 gaa ggt gga ggt tca gat gaa gtc gat tca gga gga ggt gga tct 48 Glu Gly Gly Gly Ser Asp Glu Val Asp Ser Gly Gly Gly Ser gga ggt ggc gga tee tte gag eeg tee gaa aet etg ate aet aec gtt 96 Gly Gly Gly Ser Phe Glu Pro Ser Glu Thr Leu Ile Thr Thr Val 25 gaa tog aac tog agt tgg tgg act aac tgg gtt atc cot gcg atc tot Glu Ser Asn Ser Ser Trp Trp Thr Asn Trp Val Ile Pro Ala Ile Ser . 40 195 gct ctg gtt gta gcg ctg atg tac cgg cgt taatgactgc agtctagagg g Ala Leu Val Val Ala Leu Met Tyr Arg Arg 50

<210> 2 <211> 58 <212> PRT

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<220>
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                                              ' Gly Gly Gly Ser
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Glu Ser Asn Ser Ser Trp Trp Thr Asn Trp Val Ile Pro Ala Ile Ser
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                                                45
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                                                                      48
Glu Gly Gly Gly Ser Asp Glu Val Asp Ser Gly Gly Gly Ser
                                    10
gga ggt ggc gga tcc ttc gag ccg tcc gaa act ctg atc act acc gtt
                                                                      96
Gly Gly Gly Ser Phe Glu Pro Ser Glu Thr Leu Ile Thr Thr Val
            20
                                25
gaa tcg aac tcg agt atg gac cta gga aaa ttc tgc ggg ctt tgt gtg
Glu Ser Asn Ser Ser Met Asp Leu Gly Lys Phe Cys Gly Leu Cys Val
        35
                                                45
tgt ccc tgt aac aag ctt aaa tcc agt gat gct tac aaa aaa gcc tgg
                                                                     192
Cys Pro Cys Asn Lys Leu Lys Ser Ser Asp Ala Tyr Lys Lys Ala Trp
    50
                                                                     240
ggc aat aat cag gat gga gta gtg gcc agc cag cct gcc cgt gtg gtg
Gly Asn Asn Gln Asp Gly Val Val Ala Ser Gln Pro Ala Arg Val Val
                                                                     288
gat gaa egg gag cag atg gee ate agt ggt gge tte ate ege aga ege
Asp Glu Arg Glu Gln Met Ala Ile Ser Gly Gly Phe Ile Arg Arg Arg
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gtc taa
                                                                     294
Val
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<210> 4

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Cys Pro Cys Asn Lys Leu Lys Ser Ser Asp Ala Tyr Lys Lys Ala Trp
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Asp Glu Arg Glu Gln Met Ala Ile Ser Gly Gly Phe Ile Arg Arg
Val
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                                                                     48
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99a 99t 99c 9ga too tto gag cog too gaa act otg ato act acc 9tt
                                                                     96
Gly Gly Gly Ser Phe Glu Pro Ser Glu Thr Leu Ile Thr Thr Val
            20
gaa tog aac tog agt atg gac cta gga aaa tto tgc ggg ott tgt gtg
                                                                    144
Glu Ser Asn Ser Ser Met Asp Leu Gly Lys Phe Cys Gly Leu Cys Val
       35
tgt ccc tgt aac aag ctt aaa tcc agt gat gct tac aaa aaa gcc tgg
                                                                    192
Cys Pro Cys Asn Lys Leu Lys Ser Ser Asp Ala Tyr Lys Lys Ala Trp
ggc aat aat cag gat gga gta gtg gcc agc cag cct gcc cgt gtg gtg
                                                                    240
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Gly Asn Asn Gln Asp Gly Val Val Ala Ser Gln Pro Ala Arg Val Val
 gat gaa cgg gag cag atg gcc atc agt ggt ggc ttc atc cgc aga cgc
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 Asp Glu Arg Glu Gln Met Ala Ile Ser Gly Gly Phe Ile Arg Arg
                                    90
 gtc taa
                                                                     294
                                     4/10
 Val
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Cys Pro Cys Asn Lys Leu Lys Ser Ser Asp Ala Tyr Lys Lys Ala Trp
    50
                        55
                                           60
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                    70
Asp Glu Arg Glu Gln Met Ala Ile Ser Gly Gly Phe Ile Arg Arg Arg
Val -
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                                                                     48
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gga ggt ggc gga tcc atg aca gat gcc gct gtg tcc ttc gcc aag gac
                                                                    96
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Gly	Gly	Gly	Gly 20	Ser	Met	Thr	Asp	Ala 25	Ala	Val	Ser	Phe	Ala 30	Lys	Asp		
ttc Phe	ttg Leu	gcc Ala 35	ggt Gly	gga Gly	gtg Val	gcc Ala	gca Ala 40	gcc Ala	atc Ile	tcc Ser	aag Lys	aca Thr 45	gcg	gta Val	gca Ala		144
ccc Pro	atc Ile 50	gag Glu	agg Arg	gtc Val	aag Lys	ctg Leu 55	ctg Leu	ctg Leu	5/	10	90 وعتا ا	cat His	gcc Ala	ago Ser	aag Lys		192
caa Gln 65	atc Ile	acg Thr	gca Ala	gat Asp	aag Lys 70	caa Gln	tac Tyr	aag Lys	ggc Gly	atc Ile 75	ata Ile	gac Asp	tgc Cys	gtg Val	gtt Val 80		240
cgt Arg	atc Ile	ccc Pro	aag Lys	gaa Glu 85	cag Gln	gga Gly	gtc Val	ctg Leu	tcc Ser 90	ttc Phe	tgg Trp	cgt Arg	ggg	aac Asn 95	ctg Leu	:	288
gcc Ala	aat Asn	gtc Val	atc Ile 100	aga Arg	tac Tyr	ttc Phe	ccc Pro	acc Thr 105	cag Gln	gct Ala	ctc Leu	aac Asn	ttt Phe 110	gcc Ala	ttc Phe	•	336
aaa Lys	gat Asp	aaa Lys 115	tac Tyr	aag Lys	cag Gln	atc Ile	ttt Phe 120	ctg Leu	ggt Gly	ggt Gly	gtg Val	gac Asp 125	aag Lys	agg Arg	acc Thr	·	384
cag Gln	ttc Phe 130	tgg Trp	cgc Arg	tac Tyr	ttt Phe	gca Ala 135	gjå aaa	aac Asn	ctg Leu	gca Ala	tca Ser 140	ggt Gly	ggt Gly	gcc Ala	gct Ala		432
999 Gly 145	Ala	aca Thr	tcc Ser	ttg Leu	tgc Cys 150	Phe	gtg Val	tac Tyr	cct Pro	ctt Leu 155	gat Asp	ttt Phe	gcc Ala	cgt Arg	acc Thr 160		480
cgt Arg	cta Leu	gca Ala	gct Ala	gat Asp 165	gtg Val	ggc	aaa Lys	gct Ala	gga Gly 170	gct Ala	gaa Glu	agg Arg	gaa Glu	ttc Phe 175	Lys	<i>:</i>	528
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ggc Gly	ctg Leu	tac Tyr 195	caa Gln	Gly	ttt Phe	aat Asn	gtg Val 200	tca Ser	gta Val	cag Gln	ggc Gly	att Ile 205	atc Ile	atc	tac Tyr		624
cga Arg	gct Ala 210	gcc Ala	tac Tyr	ttt Phe	ggt Gly	atc Ile 215	tat Tyr	gac Asp	act Thr	gca Ala	aag Lys 220	gga Gly	atg Met	ctc Leu	cca Pro		672
gat Asp 225	ccc Pro	aag Lys	aat Asn	act Thr	cac His 230	atc Ile	ttc Phe	atc Ile	agc Ser	tgg Trp 235	atg Met	att Ile	gca Ala	cag Gln	tct Ser 240		720
gtc Val	act Thr	gct Ala	gtc Val	gct Ala 245	ggc Gly	ctg Leu	act Thr	tcc Ser	tat Tyr 250	cct Pro	ttt Phe	gac Asp	acg Thr	gtt Val 255	cgc Arg		768
cgt Arg	cgt Arg	atg Met	atg Met	atg Met	cag Gln	tct Ser	gga Gly	cgc Arg	aaa Lys	gga Gly	act Thr	gat Asp	atc Ile	atg Met	tac Tyr		816

864

912

960

aca ggc acg ctt gac tgc tgg cgg aag atc gcg cgc gat gaa ggg agc Thr Gly Thr Leu Asp Cys Trp Arg Lys Ile Ala Arg Asp Glu Gly Ser 280 aag get ttt tte aag gge gea tgg tee aae gtt ete aga gge atg ggt Lys Ala Phe Phe Lys Gly Ala Trp Ser 6/10 I Arg Gly Met Gly 290 ggc gcc ttt gtg ctt gtc ttg tat gat gag atc aag aaa tac aca taa Gly Ala Phe Val Leu Val Leu Tyr Asp Glu Ile Lys Lys Tyr Thr 310 <210> 8 <211> 319 <212> PRT <213> Artificial sequence <223> Caspase 3 probe DEVD-ANT-2 <400> 8 Glu Gly Gly Gly Ser Asp Glu Val Asp Ser Gly Gly Gly Ser 10 Gly Gly Gly Ser Met Thr Asp Ala Ala Val Ser Phe Ala Lys Asp 20 25 30 Phe Leu Ala Gly Gly Val Ala Ala Ile Ser Lys Thr Ala Val Ala Pro Ile Glu Arg Val Lys Leu Leu Gln Val Gln His Ala Ser Lys 55 Gln Ile Thr Ala Asp Lys Gln Tyr Lys Gly Ile Ile Asp Cys Val Val Arg Ile Pro Lys Glu Gln Gly Val Leu Ser Phe Trp Arg Gly Asn Leu Ala Asn Val Ile Arg Tyr Phe Pro Thr Gln Ala Leu Asn Phe Ala Phe 105 Lys Asp Lys Tyr Lys Gln Ile Phe Leu Gly Gly Val Asp Lys Arg Thr Gln Phe Trp Arg Tyr Phe Ala Gly Asn Leu Ala Ser Gly Gly Ala Ala 130 135 Gly Ala Thr Ser Leu Cys Phe Val Tyr Pro Leu Asp Phe Ala Arg Thr Arg Leu Ala Ala Asp Val Gly Lys Ala Gly Ala Glu Arg Glu Phe Lys Gly Leu Gly Asp Cys Leu Val Lys Ile Tyr Lys Ser Asp Gly Ile Lys 185

1		19	5	14 GI	у Бп	e ası	200		r va	1 G11	n GI	20 A 11		le II	le Ty	rī	
Arg	Al. 21	a Al O	а Ту	yr Ph	e Gl	y Ile 215	e Tyı	r Asj	p Th	r Ala	22		у Ме	t Le	eu Pr	Ò	
Asp 225	Pro	o Ly	s As	n Th	r. Hi 23	s Ile O	Phe	∋ Ile		r Tro /10	Me	t Il	e Al	a ·G]	n Se 24		
Val	Thi	r Ala	a Va	al Ala 24	a Gl; 5	y Leu	Thr	Ser	r Ty:	r Pro	Phe	e Ası	p Th	r Va 25	l Ar	g	
Arg	Arg	Me	t Me 26	t Me	t Gl	n Ser	Gly	265	J Lys	s Gly	Thi	r Ası	27		t Ty	r'	
Thr	Gly	7 Thi 27!	r Le	u Asj	o Cy	s Trp	Arg 280	Lys	; Ile	Ala	Arg	Asp 285		u Gl	y Ser	r	
Lys	Ala 290	Phe	e Ph	e Lys	s Gly	Ala 295	Trp	Ser	Asn	val	Leu 300	Arg	, Gl	y Me	t Gly		•. ••
Gly 305	Ala	Phe	e Va	l Lei	1 Val	Leu)	Tyr	Asp	Glu	Ile 315		Lys	ТУ	r Th	r		٠
<210 <211 <212	> 4 !> D	11 NA										· .					
<213	> A	rtif	ici	al s∈	quen	ice									:		
<220 <223		aspa	se 3	3 prc	be H	2B-D	EVD								٠		
<220				•													
<221 <222			(411	L) ;			•		•								
<400		~ ~~														•	-
Met 1	Pro	Glu	Pro	Ala 5	Lys	Ser	Ala	Pro	gcc Ala 10	Pro	aaa Lys	aag Lys	ggc	Ser 15	aag Lys	•	4.8
aag (Lys i	gcg Ala	gtg Val	act Thr 20	rys aag	gcg Ala	cag Gln	Lys aag	aaa Lys 25	ggc ggc	ggc Gly	aag Lys	aag Lys	cgc Arg 30	aag Lys	egc Arg		96
agc (Ser)	cgc Arg	aag Lys 35	gag Glu	agc Ser	tat Tyr	tcc Ser	atc Ile 40	tat Tyr	gtg Val	tac Tyr	aag Lys	gtt Val 45	ctg Leu	aag Lys	cag Gln		144
val r	cac lis	cct Pro	gac Asp	acc Thr	ggc	att Ile 55	tcg Ser	tcc Ser	aag Lys	gcc Ala	atg Met 60	ggc Gly	atc Ile	atg Met	aat Asn	•	1 92
cg t Ser E	tt Phe	gtg Val	aac Asn	gac Asp	att Ile 70	ttc Phe	gag Glu	cgc Arg	Ile	gca Ala 75	ggt Gly	gag Glu	gct Ala	tcc Ser	cgc Arg 80		240
tg g eu A	gcg Ma	cat His	tac Tyr	aac Asn 85	aag Lys	cgc Arg	tcg Ser	Thr	atc Ile 90	acc Thr	tcc Ser	agg Arg	gag Glu	atc Ile 95	Cag Gln		288

336

384

411

acg gcc gtg cgc ctg ctg cct ggg gag ttg gcc aag cac gcc gtg Thr Ala Val Arg Leu Leu Pro Gly Glu Leu Ala Lys His Ala Val 105 tcc gag ggt act aag gcc atc acc aag tac acc agc gct aag gat cca Ser Glu Gly Thr Lys Ala Ile Thr Lys Tvr Thr Ser Ala Lys Asp Pro 115 120 8/10 ccg gtc gat gaa gtc gat gcc acc atg Pro Val Asp Glu Val Asp Ala Thr Met 130 <210> 10 <211> 137 <212> PRT <213> Artificial sequence <223> Caspase 3 probe H2B-DEVD <400> 10 Met Pro Glu Pro Ala Lys Ser Ala Pro Ala Pro Lys Lys Gly Ser Lys Lys Ala Val Thr Lys Ala Gln Lys Lys Gly Gly Lys Lys Arg Lys Arg Ser Arg Lys Glu Ser Tyr Ser Ile Tyr Val Tyr Lys Val Leu Lys Gln 35 Val His Pro Asp Thr Gly Ile Ser Ser Lys Ala Met Gly Ile Met Asn Ser Phe Val Asn Asp Ile Phe Glu Arg Ile Ala Gly Glu Ala Ser Arg 70 Leu Ala His Tyr Asn Lys Arg Ser Thr Ile Thr Ser Arg Glu Ile Gln 90 Thr Ala Val Arg Leu Leu Pro Gly Glu Leu Ala Lys His Ala Val 105 Ser Glu Gly Thr Lys Ala Ile Thr Lys Tyr Thr Ser Ala Lys Asp Pro Pro Val Asp Glu Val Asp Ala Thr Met 130 <210> 11 <211> 414 <212> DNA <213> Artificial sequence <220>

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48

96

144

192

240

288

336

384

<220>

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 Lys Ala Val Thr Lys Ala Gln Lys Lys Gly Gly Lys Lys Arg Lys Arg
                                                     30
 age ege aag gag age tat tee ate tat gtg tae aag gtt etg aag eag
 Ser Arg Lys Glu Ser Tyr Ser Ile Tyr Val Tyr Lys Val Leu Lys Gln
 gtc cac cct gac acc ggc att tcg tcc aag gcc atg ggc atc atg aat
 Val His Pro Asp Thr Gly Ile Ser Ser Lys Ala Met Gly Ile Met Asn
                         55
 tcg ttt gtg aac gac att ttc gag cgc atc gca ggt gag gct tcc cgc
 Ser Phe Val Asn Asp Ile Phe Glu Arg Ile Ala Gly Glu Ala Ser Arg
 ctg gcg cat tac aac aag cgc tcg acc atc acc tcc agg gag atc cag
 Leu Ala His Tyr Asn Lys Arg Ser Thr Ile Thr Ser Arg Glu Ile Gln
               . 85
                                     90
acg gcc gtg cgc ctg ctg cct ggg gag ttg gcc aag cac gcc gtg
 Thr Ala Val Arg Leu Leu Leu Pro Gly Glu Leu Ala Lys His Ala Val
             100 -
                                 105
tcc gag ggt act aag gcc atc acc aag tac acc agc gct aag gat cca
 Ser Glu Gly Thr Lys Ala Ile Thr Lys Tyr Thr Ser Ala Lys Asp Pro
                             120
                                               125
ccg gtc gtc gtc gcc gat gcc acc atg
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Ser Arg Lys Glu Ser Tyr Ser Ile Tyr Val Tyr Lys Val Leu Lys Gln
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Val His Pro Asp Thr Gly Ile Ser Ser Lys Ala Met Gly Ile Met Asn
Ser Phe Val Asn Asp Ile Phe Glu Arg Ile Ala Gly Glu Ala Ser Arg
Leu Ala His Tyr Asn Lys Arg Ser Thr Ile Thr Ser Arg Glu Ile Gln
                                   10/10
Thr Ala Val Arg Leu Leu Pro Gly Glu Leu Ala Lys His Ala Val
Ser Glu Gly Thr Lys Ala Ile Thr Lys Tyr Thr Ser Ala Lys Asp Pro
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gga ggt ggc gga tcc ttc gag ccg tcc gaa act ctg atc act acc gtt
                                                                     96
Gly Gly Gly Ser Phe Glu Pro Ser Glu Thr Leu Ile Thr Thr Val
            20
                               25
gaa tog aac tog agt tgg tgg act aac tgg gtt atc oot gog atc tot
                                                                    144
Glu Ser Asn Ser Ser Trp Trp Thr Asn Trp Val Ile Pro Ala Ile Ser
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gct ctg gtt gta gcg ctg atg tac cgg cgt taa
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Gly Gly Gly Ser Phe Glu Pro Ser Glu Thr Leu Ile Thr Thr Val 20 25 30

Glu Ser Asn Ser Ser Trp Trp Thr Asn Trp Val Ile Pro Ala Ile Ser 35 40 45

Ala Leu Val Val Ala Leu Met Tyr Arg Arg 50 55